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## THE COMPLEMENT FIXATION TEST FOR SYPHILIS.

(MODIFIED WASSERMANN.)

DESCRIPTION OF A METHOD AT PRESENT IN USE AT THE HYGIENIC LABORATORY.

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In view of the increasing use of the complement fixation test for syphilis in connection with efforts to prevent venereal diseases throughout the country, the following statement has been prepared to describe a method of performing this test now in use at the Hygienic Laboratory of the United States Public Health Service.

### Apparatus, Specifications and Descriptions.

Specifications for the laboratory apparatus specially needed are given below. Where quantities are stated (figures in parentheses) the estimate is based on the performance of 100 tests at a time two or three times a week, allowing an interval for cleaning and sterilizing apparatus for the next tests. Where articles are solely for use in the preparation of antigen and hæmolytic amboceptor a note to this effect follows.

Test-tube racks, water baths, and an ice box superior to the ordinary refrigerator in the maintenance of a low temperature may readily be constructed by the tinsmith and carpenter at a saving over the market prices.

An autoclave or Arnold sterilizer and dry (hot air) sterilizer, a balance, materials for cleaning glassware, etc., form part of the equipment of any bacteriologic laboratory and do not need to be specially described. Following are detailed specifications of articles used in connection with the Wassermann tests.

Burner (for water bath):

Micro, 2½ inches high, with long stem (1).

Centrifuge: High speed, electric drive (1).

Type, International Equipment Co. Complete with 4-tube 50 cc. head and accessories, and 4-tube 15 cc. head and accessories.

Centrifuge tubes:

15 cc. ungraduated (12).

50 cc. for International Equipment Co. centrifuge (6).

Flasks, filtering, Erlenmeyer shape, of heavy glass to withstand pressure, with side neck, 2,000 cc. capacity (2) (for making antigen only).

Flasks, Erlenmeyer pyrex glass:

100 cc. (6).

500 cc. (6).

Glass cylinders with ground-glass stoppers, single graduations:

25 cc. (3).

100 cc. (3).

250 cc. (3).

Ice box:

A straight-sided earthenware crock, about a foot high and of about that diameter, is placed inside a packing box about 2 feet square. Sawdust is packed around the crock, filling the box so that the top of the crock is flush with the top of the box. Inside the crock is placed a pail of about two-thirds the diameter of the crock, and cracked ice is packed between the pail and the crock. For a cover, a section cut out of the wooden box above the pail completes the outfit. Reagents placed in the pail may be preserved at a temperature of 4° C. if care be taken to maintain the supply of ice.

Needles, hollow, nickel-plated Luer slips:

Gauge 18 (6).

Gauge 23 (6) (for intravenous inoculation of rabbits for amboceptor production).

Syringe, all-glass, Luer type, 10 cc. capacity (2).

Syringe, all-glass, Luer type, 20 cc. capacity (2).

Test tubes, 120 mm. by 16 mm., of clear white, noncorrosive glass (230).

Test glasses, conical shape, low, wide form, with broad flat bottom. Capacities:

50 cc. (6).

100 cc. (6).

250 cc. (6).

Test-tube baskets, of brass wire, rectangular shape, opening 120 by 100 mm., 130 mm. deep (5).

Test-tube racks (2), constructed of sheet copper or galvanized iron, about 24 gauge, dimensions 17½ inches long, 5½ inches wide, 2½ inches high.

The rack consists essentially of 3 pieces of sheet metal placed one above the other, the top sheet 2½ inches above the bottom and the middle sheet one-half inch above the bottom one. These are securely fastened together at the ends by bent-up extensions of the bottom piece, to which the other portions of the rack are riveted. The two upper sheets are perforated with 6 rows of 16 holes, $\frac{11}{16}$-inch or slightly larger, large enough to hold the test tubes. Bent wire handles attached at each end of the rack are a convenience.

Thermometers, chemical, long stem, centigrade scale, −10 to +110° (4).

Thermo regulator, Roux bimetallic, length 10 inches (1).

Pipettes, Mohr type:

Capacity 10 cc., graduated in $\frac{1}{10}$ cc. (10).

Capacity 5 cc., graduated in $\frac{1}{10}$ cc. (10).

Capacity 2 cc., graduated in $\frac{1}{10}$ cc. (10).

Capacity 1 cc., graduated in $\frac{1}{10}$ cc. (120).

Graduation marks on pipettes should not extend nearer than 3½ inches to the end opposite the tip.

Pipette boxes 16 inches long, 2½ inches diameter (for holding pipettes during sterilization), cylindrical in form, of copper with tightly fitting lid: (3)

Water bath:

A rectangular tank 18 inches long, 13 inches wide, and 8 inches in diameter is made water-tight, of sheet metal, about 20-gauge copper or galvanized iron. It is open at the top and fitted with a suitable cover. The bath is supported in

any suitable way so as to allow about 10 inches space beneath it. Inside the bath is placed a rack of galvanized-iron wire 2 or 3 inches from the bottom. At one corner of the bath is fitted a Roux bimetallic thermoregulator with rubber connection to the gas cock, and the microburner which is placed under the bath. The bath is filled two-thirds full of water at about 37.5° C. and the regulator adjusted till this temperature is constantly maintained, as shown by a thermometer kept in the bath.

Water pump (vacuum):

Filter type (1) (for making antigen).

Preparation of glassware:

Cleaning. All new glassware should be cleaned by immersion in a mixture of potassium bichromate, 120 gm.; water, 800 cc.; concentrated sulphuric acid, 600 cc. Pulverize the bichromate and dissolve in the water. Then add the sulphuric acid slowly. After removal from this mixture the apparatus must be thoroughly rinsed to remove every trace of acid. After use, glassware should be thoroughly rinsed in *cold* water to remove serum or blood, *then* scrubbed with soap-powder solution, rinsed thoroughly with running water, and finally with distilled water. Test tubes should be packed, inverted, in a basket and thoroughly dried in the hot-air oven. Pipettes may conveniently be sterilized in copper pipette boxes.

All other articles of glass should be sterilized by heat, except the glass measuring cylinders, which are easily broken. These should be made chemically clean and rinsed with 0.9 per cent sodium chlorid solution before use. In serological work sterility is desirable; chemical cleanliness of all glassware is essential.

## Reagents, Special Substances Used in the Tests.

### PREPARATION AND PRESERVATION.

*Sodium chlorid solutions.*—A nine-tenths per cent solution of the chemically pure salt in distilled water is made up by weight, distributed in 500 cc. Erlenmeyer flasks and sterilized by steam.

*Saturated sodium chlorid solution.*—Is made by saturating a small sterile bottle of distilled water with the chemically pure salt. The bottle is to be kept at room temperature.

*Sheep's blood corpuscles.*—These are best obtained by bleeding a sheep from the jugular vein by use of a sterile syringe, previously rinsed with saline solution, transferring the blood immediately to a sterile 50 cc. Erlenmeyer flask containing sterile glass fragments, and agitating for 15 minutes, avoiding foaming, thus defibrinating the blood. Sheep's corpuscles can also be obtained at the abattoir by catching the blood coming from the vessels of the sheep's neck directly in the defibrinating flask. In doing this due care should be taken to avoid the entrance of gross dirt into the flask. After defibrinating, decant the blood into a graduated cylinder which is either perfectly dry or has just been rinsed out with 0.9 per cent sodium chlorid solution. Note the amount of blood and divide it about equally between two or more 50-cc. centrifuge tubes. Add 0.9 per cent sodium chlorid solution till tubes are nearly full, and mix thoroughly. The glass centifuge tubes in place within the metal tubes and trunnion rings in place should

be balanced against each other in pairs. Centrifuge till the corpuscles are completely precipitated. Pipette off supernatant fluid, add fresh 0.9 per cent sodium chlorid solution, mix thoroughly, centrifuge again, pipette off the supernatant fluid, add fresh sodium chlorid solution, mix, and again centrifuge. After the third centrifuging, pipette off the fluid above the blood cells, transfer the cells to the cylinder previously used for measuring the volume of the blood, which should be freshly rinsed with saline solution, and make up to the original volume with 0.9 per cent sodium chlorid solution. Keep blood corpuscles in the ice box at a temperature not above 6°. Cells may thus be preserved for at least 48 hours. The blood cells should not be used if the supernatant sodium chlorid solution shows a reddish coloration. If after the final washing a sterile 5 per cent dextrose solution be used to make the corpuscle suspension up to the original volume of the blood, the corpuscles should show no evidence of disintegration at the end of 10 days, if kept at a temperature of not more than 6°. Under these conditions they may be used in the test the same as fresh cells.

*Hæmolytic amboceptor.*—This substance is produced in the serum of a rabbit by injecting this animal with the washed blood corpuscles of a sheep prepared as above. The following methods of producing hæmolytic amboceptor may be used:

1. Inject rabbits intravenously with 1 cc. of fresh sheep cell suspension on the first, fourth, seventh, and tenth days of the process. Make test bleeding on the fifteenth day.

2. Inject rabbits intravenously with 1 cc., 1 cc., and 2 cc. washed sheep corpuscles on three successive days. Wait five days and repeat the injections. Make test bleeding five days after the last injection.

When the rabbit sera have been found to contain hæmolytic amboceptor of a suitable strength, the animals should be exsanguinated into large sterile centrifuge tubes or test tubes. When the blood has clotted, separate the clot from the sides of the tube by means of a platinum wire, or glass rod, and place the tubes in the 37° water bath for an hour, then place in the cold box over night. Separate the serum, add 0.3 per cent phenol, and preserve in the ice box at a temperature not higher than 6° C. Amboceptor serum should be stored two weeks before use.

*Complement.*—Bleed not less than five full-grown guinea pigs from the heart by means of a sterile syringe, previously rinsed with salt solution. With proper technique, from 5 to 10 cc. may be obtained without injury to the animal. Pigs may be kept for this purpose and bled once in two weeks. Place the blood drawn from each pig in a separate centrifuge tube, and allow to clot. Separate the clot from the side of the tubes with sterile needle or pipette and place in 37° water bath for one hour. Then place in cold box overnight, or, if serum has separated, pipette off the serum immediately. Centri-

fuge clear of red blood corpuscles and pool the sera in a sterile glass container. For each cubic centimeter of pooled sera add one-tenth cubic centimeter saturated sodium chlorid solution, mix well, and preserve in the cold box at a temperature not above 6° C. Guinea-pig serum so preserved will retain its complement undiminished in hæmolytic properties for two weeks. Just before use dilute with three volumes of distilled water, to restore the normal tonicity, and dilute as convenient with 0.9 per cent sodium chlorid solution.

*Antigen.*—A suspension of ether and alcohol soluble, acetone insoluble lipoids is used in the test. This is prepared as follows: A fresh beef heart is freed from fat and connective tissue, and the muscle ground in a meat grinder. Place 200 gm. of the ground meat in a bottle, add 2,000 cc. absolute alcohol, and stopper tightly. Extract for 10 days or 2 weeks at 37° C., thoroughly shaking the bottle three times each day. Filter through filter paper, place the filtrate in a filtering flask, and attach to an exhaustion apparatus. Agitate from time to time, heating the flask on a 37° C. water bath. When the contents of the flask have been evaporated to dryness, wash out the residue with about 100 cc. ether, evaporate the ether solution to about 30 cc., place in a conical cover and set aside in a cool place over night, decant clear supernatant fluid and add slowly to it ten times the volume of acetone to the filtrate, stir, and set aside, covered, in a cold place, for the precipitate to form. Collect precipitate, bottle with a little acetone, and preserve in the cold box. For use, dissolve 0.3 gm. of the solid in 1 cc. ether and 9 cc. of best obtainable grade of methyl alcohol. Preserve in the cold box at a temperature not above 6° C. Mix with 9 per cent sodium chlorid solution as indicated by the antigenic titration (see below) for use in the tests.

*The patient's serum.*—Blood may be obtained from the arm vein. To do this sterilize a syringe and its needle by boiling, and also sterilize the skin of the front of the arm at the bend of the elbow. Rinse the syringe with saline solution. After the patient has opened and closed the fist vigorously several times, to pump the blood into the veins, place a tourniquet above the elbow just tightly enough to cut off the venous circulation. Puncture a prominent vein with the needle; draw up 5 cc. blood. Loosen the tourniquet and discharge the blood into a 15-cc. centrifuge tube. The venous puncture ordinarily requires no dressing. The procedure is best carried out with the patient lying down. When the serum has clotted, separate the clot from the side of the tube, and set in a cool place, to allow the clot to contract. When the serum has separated, pipette off and transfer to another sterile glass container till tested. Keep in the ice box at a temperature not above 6° C. Sera should always be separated from the clot before shipment, as if this is not done more or less

hæmolysis will take place en route, rendering the serum unfit for testing.

Serum should be shipped in a sealed glass capsule, or small, sterile, rubber-stoppered bottle. The stopper should be firmly inserted and a strip of adhesive plaster pasted over the top to guard against its being dislodged. Blood serum in sealed glass capsules, or sterile vials, should be well wrapped in cotton and placed in a double mailing case, as specified by the postal regulations. (See reprint from Public Health Reports No. 438.)

Just before subjecting the sera to the Wassermann test they should be heated in a water bath at 54° to 56° C. for one-half hour, but spinal fluids do not require heating. The sera should be fresh—i. e., not more than 24 hours old. Tests may be performed with sera older than this, but in that case more negative results with the sera of syphilitics are to be anticipated than if the sera were fresh.

STANDARDIZATION OF REAGENTS, TITRATING.

The Wassermann reaction, properly performed, is a quantitative biochemical reaction, our knowledge of which is wholly empirical. To perform it properly, the various quantities of the elements entering into it should be measured as precisely as possible. Furthermore, it is evident that the substances used are extremely complex organic materials, and the greatest possible care should be taken to handle them properly and measure them accurately. A word may be said here about the use of the graduated pipettes, in measuring reagents diluted or undiluted.

To measure 0.1 cc. use a 1 cc. pipette graduated in tenths.

To measure 0.2 cc. use a 1 cc. pipette graduated in tenths.

To measure from 0.3 cc. to 1 cc. use a 1 cc. or 2 cc. pipette graduated in tenths.

To measure 1 cc., or multiples thereof, use a 5 cc. or 10 cc. pipette graduated in tenths.

In measuring sodium chlorid solution to make up the contents of tubes to unit volume, a 5 cc. pipette graduated in tenths may be used to measure quantities of 0.5 cc. and more.

The necessity for conscientious accuracy in the use of pipettes can not be over emphasized. In measuring reagents the direct measurements of minute amounts (less than 0.1 cc.) is to be avoided. Such amounts should be measured indirectly by diluting the reagent with 0.9 per cent sodium chlorid solution and measuring a portion of the resulting solution corresponding to the desired amount of the undiluted reagent. In making dilutions the conical test glasses are convenient, and thorough mixing may be secured by blowing air through the solutions. It is needless to remark, after measuring one reagent the pipette should be discarded and a fresh one used before measuring another reagent.

The substances first requiring attention are sheep's corpuscles, hæmolytic amboceptor (rabbit) serum, and complement (guinea pig) serum. The proper adjustment of these substances in relation to each other, known as the adjustment of the hæmolytic system, is an essential preliminary to the tests for syphilis.

*Sheep's blood corpuscles.*—The sheep cell suspension previously described is added to 0.9 per cent sodium chlorid solution in the proportion of 5 cc. of the suspension to 95 cc. of the saline solution. (For details of preparation see amboceptor titration and complement titration.) This suspension is taken as an arbitrary starting point in measuring the amount of hæmolytic amboceptor present in the rabbit serum, and the quantity of complement present in the guinea pig serum to determine the proper amounts of these substances to use in the tests.

It should always be remembered, however, that the red blood corpuscles of different sheep vary considerably in the ease with which they are hæmolyzed by complement and amboceptor; so that the substitution of the corpuscles of one sheep for those of another may cause an actual variation of as much as 100 per cent in the quantity of amboceptor or complement serum necessary to cause complete hæmolysis, and thus give the appearance of a sudden change in the potency of these reagents. This variability of the sheep corpuscles is taken into account and provided for, as is the variability of other reagents, by daily titration of complement just before setting up the tests for syphilis.

*Titration of hæmolytic amboceptor.*—The "unit of amboceptor" is the smallest amount of amboceptor serum which with 0.05 cc. fresh pooled guinea pig serum will completely hæmolyse 1.0 cc. of the 5 per cent suspension of sheep cells, when exposed to a temperature of 37° C. for one hour.

Select a specimen of antisheep rabbit serum at least two weeks old. Place 0.1 cc. in a conical glass and add precisely 9.9 cc., 0.9 per cent sodium chlorid solution (i. e., 1 in 100 dilution); mix thoroughly by blowing air through the solution; take 0.5 cc. of this and add 9.5 cc., 0.9 per cent sodium chlorid solution (i. e., 1 in 2,000 dilution); then each cubic centimeter of the final dilution will contain 0.005 cc. rabbit serum. Now place the following amounts of the final dilution in a row of test tubes:

0.1 cc. containing 0.0005 cc. amboceptor serum.
0.2 cc. containing 0.001 cc. amboceptor serum.
0.3 cc. containing 0.0015 cc. amboceptor serum.
0.4 cc. containing 0.002 cc. amboceptor serum.
0.5 cc. containing 0.0025 cc. amboceptor serum.
0.6 cc. containing 0.003 cc. amboceptor serum.

Add one tube, containing no amboceptor serum, to the row and make up the volume in all tubes to 2 cc. with 0.9 per cent sodium chlorid solution. Take 1 cc. of the pooled sera of at least 5 guinea pigs, which has been obtained within 5 hours and kept cold, the serum to be unsalted, and add 19 cc. 0.9 per cent sodium chlorid solution. Each cc. will therefore contain 0.05 cc. of the guinea pig serum. Add 1 cc. of the diluted guinea pig serum containing complement to all the test tubes. Next add to all the tubes a 5 per cent dilution in saline solution of the sheep cell suspension already described, making the total volume in each tube 4 cc., mix thoroughly by agitating the tubes, place in the 37° water bath for one hour, and keep at about 15° C. overnight. Note the tube containing the least amount of hæmolytic amboceptor serum which shows complete hæmolysis. By complete hæmolysis is meant a cloudless red solution with no undissolved corpuscles at the bottom of the tube. The amount of rabbit serum in this tube is the "unit of amboceptor." Reject, as unsuitable, those specimens of rabbit serum which fail to give complete hæmolysis in amounts of 0.002 cc. or less, with 0.05 cc. pooled complement sera. Amboceptor serum should be retitrated every six weeks. In titrating a new specimen of amboceptor serum set up a duplicate test, using a specimen of amboceptor serum of known titre.

*Titration of complement.*—This is to be done daily just before the syphilis tests are set up.

The "unit of complement" is the smallest amount of complement serum which, with two units of amboceptor, will completely hæmolyse 1 cc. of the 5 per cent sheep cell suspension when kept at a temperature of 37° C. for one-half hour.

Estimate, in round numbers, the number of cubic centimeters of red cell suspension needed for the day's work; for example, 100 cc. Multiply the unit of amboceptor by 200 and place that amount of amboceptor serum in a 100 cc. glass-stoppered graduated cylinder. Add about 50 cc. of 0.9 per cent sodium chlorid solution, taking care to wash down the serum adhering to the sides of the cylinder; next add 5 cc. of the undiluted sheep corpuscles which have been made up to the volume of the defibrinated blood. Then make up to 100 cc. with 0.9 per cent sodium chlorid solution. Invert 50 times to mix thoroughly. Set aside for 15 minutes.

Dilute some of the salted complement serum as follows:

| | |
|---|---|
| Serum | 0.3 cc. |
| Water | 0.9 cc. |
| 0.9 per cent solution sodium chlorid | 1.8 cc. |
| Total | 3.0 cc. |

Set up seven test tubes, adding the following amounts of the above solution to them: 0.6 cc., 0.5 cc., 0.4 cc., 0.3 cc., 0.2 cc., 0.1 cc., 0 cc., using a 1 cc. pipette. Make up the volume in each tube

with saline solution to 3 cc. Use a 1 cc. pipette to make up tenths and a 5 or 10 cc. pipette to add the necessary 2 cc. Add to each tube 1 cc. of the amboceptor-corpuscle suspension, incubate in a water bath at 37° C. for one-half hour, and read the unit at once by noting the tube containing the least amount of guinea-pig serum in which the cells are completely dissolved.

*Titration of antigen.*—In determining the suitability and amount of a specimen of the acetone-insoluble lipoids for use as antigen, the following properties of this substance especially concern us.

1. The property of the antigen of combining with complement in the presence of syphilitic sera.

2. The property of the antigen, in much larger amounts, of combining with complement in the presence of normal sera.

3. The property of the antigen of hæmolysing the red blood cells.

The first two properties are present to a degree in nearly all antigens, while the third occurs only occasionally and is reason for the rejection of the particular specimen in question.

The quantitative estimation of the first is called the antigenic titration; that of the second the anticomplementary titration. These processes may be combined as follows: Set up two parallel rows of 12 tubes and add to them, in pairs, graded amounts of methyl alcohol solution of the antigen to be tested, leaving one pair without antigen for control—viz, 0.2, 0.16, 0.14, 0.1, 0.08, 0.06, 0.04, 0.02, 0.01, 0.006, 0.004, 0.000 cc. In adding the antigen solution, dilute 1 part in 10 with 0.9 per cent sodium chlorid solution. Make up to 2 cc. with 0.9 per cent sodium chlorid solution. To each tube of one row add 0.2 cc. of known positive syphilitic serum, and to each tube of the other 0.2 cc. of known negative serum. Add to each tube two units of complement, just previously determined as already described, contained in 1 cc. of 0.9 per cent sodium chlorid solution. This may conveniently be done as follows:

Total number tubes=24 (allowing for fluid lost in measurement, 25 cc. complement solution will be needed).
Unit of complement=0.03×2=0.06.
Then 0.06×25=1.50.

| | | |
|---|---|---|
| Take salted complement serum | 1.50 cc. | |
| Water | 4.5 cc. | (3×1.5) |
| | 6.0 cc. | |
| 0.9 per cent sodium chlorid solution | 19.0 cc. | (25−6=19) |
| Total | 25.0 cc. | |

Then each cubic centimeter of the solution contains two units of complement.

Mix contents of tubes thoroughly, place in 37° water bath for one hour. Remove and add to each tube 1 cc. of amboceptor sheep

corpuscles, used in determining the unit of complement. Incubate one-half hour and set in a cold place about 15° C. over night.

Note the least amount of antigen completely preventing hæmolysis in the tubes containing positive syphilitic serum. Note the largest amount of antigen not interfering with complete hæmolysis in the negative serum tubes. The best antigen gives a wide margin between these readings.

Now select the unit of antigen for use in the syphilis tests between these values. It should be several times the least amount of antigen completely preventing hæmolysis in the tubes containing positive sera; as other positive sera may be encountered, weaker in the syphilitic reacting substance than the specimen used. On the other hand, the unit should not be more than one-half the largest amount of antigen not interfering with complete hæmolysis in the tubes containing negative serum, as other negative sera may be more anti-complementary than the one used in the test, and false positive reactions might result from the use of too much antigen.

Example of selection of antigenic unit:

| With negative serum. | | With positive serum. | |
|---|---|---|---|
| Amount antigen. | Hæmolysis. | Amount antigen. | Hæmolysis. |
| 0.2 cc | None | 0.02 cc | None. |
| 0.16 cc | Partial | 0.01 cc | Do. |
| 0.14 cc | do | 0.006 cc | Do. |
| 0.1 cc | Complete | 0.004 cc | Partial. |
| 0 | do | 0 | Complete. |

Unit selected = .02 cc. (methyl alcohol solution).

When the unit has been selected, place 2 units diluted to 3 cc. in a test tube and add 1 cc. of the cell suspension. Incubate 1 hour at 37° C. Should any hæmolysis occur, the specimen of antigen should be rejected as being hæmolytic.

### The Complement-Fixation Test for Syphilis.

#### PERFORMING THE TESTS.

After the units of amboceptor and antigen have been determined, the unit of complement titrated, and the patient's sera heated to 54° – 56° C. for one half hour on the day of the test, the test may be set up as follows (see diagram): Set up a pair of tubes for each new serum to be tested and for the positive and negative control sera which are to be retested. Add one tube for the antigen control, one for the hæmolytic system control, and one for the sheep corpuscle control. The paired tubes are conveniently placed in two rows, front and back. To each of the front row tubes add one unit of antigen contained in 1 cc. of 0.9 per cent sodium chlorid solution, and

to the antigen control tube add 2 units contained in 2 cc. To dilute the methyl alcohol solution of antigen for this purpose multiply the unit by the number of sera to be examined plus five. Place this amount of the methyl alcohol antigen solution in a conical glass and add sufficient of the diluent to make the total volume equal to the number of specimens to be examined plus five.

Eg. Antigen unit=0.03.
Number of sera including positive and negative controls plus 5=40.
0.03×40=1.20.
Use 1.2 cc. antigen solution and add to it 40 cc. minus 1.2 cc., or 38.8 cc. 0.9 per cent sodium chlorid solution.

Then, each cubic centimeter of the antigen suspension as made up will contain 0.03 c. c. of the methyl alcohol solution.

Now to each pair of tubes, corresponding to the sera which are to be tested, add 0.2 cc. of the sample of serum to the front tube and 0.4 cc. to the back tube. Make the volume of all tubes equal 2 cc. with 0.9 per cent sodium chlorid solution. To do this add 2 cc. of it to the hæmolytic control tube, 3 cc. to the corpuscle control tube, 1.6 cc. to the tubes in the back row containing sera and 0.8 cc. to the tubes in the front row.

Add to all tubes, save the corpuscle control tube, 2 units of complement contained in 1 cc. 0.9 per cent sodium chlorid solution (see antigen titration). Add 1 cc. of this mixture to each tube, except the corpuscle control tube. The volume contained in each tube will now be 3 cc. Mix well, by individually agitating each tube. Incubate in a water bath at 37° C. for one hour. Add to each tube 1 cc. of the amboceptor-sheep corpuscle suspension. Mix well and incubate as above for one-half hour. Place in a cool place at about 15° C. overnight.

READING AND RECORDING RESULTS.

The morning after performing the tests, first examine the control tubes. All the red cells in the antigen and hæmolytic system control tubes should be hæmolysed, but there should be no trace of hæmolysis in the corpuscle control tube. Next examine the tubes containing known positive and negative sera. The rear tubes of both these pairs should show complete hæmolysis, as should the front tube of the pair containing negative serum. The front tube of the positive pair should, however, show little or no hæmolysis, indicating complete, or nearly complete, fixation of complement. In like manner examine all the tubes containing serum to be tested. Inspect the back tubes first; if complete hæmolysis is not present it may be concluded that the serum was anticomplementary, i. e., was capable of fixing complement in the absence of antigen, and that any fixation in the front tube is of doubtful significance. In some sera, in which hæmolysis is complete in the back tube, various degrees of

fixation, i. e., weakening of hæmolysis, will be noted in the corresponding front tubes. If the appearances of the controls previously mentioned are satisfactory, it is permissible to conclude that these sera are positive.

Record the results of the tests, as indicated by the amounts of fixation in these tubes as compared with a specimen showing complete fixation (no hæmolysis) and one showing no fixation (complete hæmolysis) reporting the results as follows:

70 to 100 per cent fixation = "strongly positive."
40 to 70 per cent fixation = "positive."
20 to 40 per cent fixation = "weakly positive."
0 to 20 per cent fixation = "negative."

In actual practice, with the technique described, the experience has been that nearly all sera give either "strongly positive" or "positive" reactions, or are frankly negative.

*Diagram of complement fixation test for syphilis.*

The squares represent the arrangement of tubes as seen by one looking down on the rack. Inside the squares appear the reagents in the order in which they are introduced together with the amounts. The preliminary phase of the incubation is carried out at 37° C. for 1 hour. Add amboceptor-cell suspension, incubate at 37° for one-half hour, and keep at about 15° C. overnight.

BACK ROW.

| Known positive serum. | Known negative serum. | Unknown serum[1] to be tested for syphilis. | Antigen control. | Hæmolytic system control. | Sheep corpuscle control. |
|---|---|---|---|---|---|
| Serum .4 cc.<br>NaCl sol. 1. 6 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | Serum .4 cc.<br>NaCl solution 1. 6 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | Serum .4 cc.<br>NaCl solution 1. 6 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | Antigen suspension 2 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | NaCl solution 2 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | NaCl solution 3 cc.<br>Amboceptor-corpuscle suspension 1 cc. |

FRONT ROW.

| | | | | | |
|---|---|---|---|---|---|
| Antigen suspension 1 cc.<br>Serum .2 cc.<br>NaCl solution .8 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | Antigen suspension 1 cc.<br>Serum .2 cc.<br>NaCl solution .8 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | Antigen suspension 1 cc.<br>Serum .2 cc.<br>NaCl solution. 8 cc.<br>Complement dilution 1 cc.<br>Amboceptor-corpuscle suspension 1 cc. | | | |

[1] But one tube shown in diagram.

## STERILIZATION OF THE MENTALLY DEFECTIVE AND INSANE.

A Michigan law providing for the sterilization of mental defectives or insane persons maintained wholly or in part by public expense in public institutions has been declared unconstitutional by the Michigan